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(54) **POLYPEPTIDE PHARMACEUTICALLY ACCEPTABLE SALT ET USE THEREOF**

(57) There is provided in the present application a pharmaceutically acceptable salt of a polypeptide and a pharmaceutical composition comprising the same, wherein the polypeptide comprises the amino acid sequence YEKLLDTEI or a functional variant thereof.

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Description

TECHNICAL FIELD

[0001] The present application generally relates to the biomedical field. In particular, there is provided in the present application pharmaceutically acceptable salts of polypeptides, compositions, and methods for treating, ameliorating, or preventing disorders associated with the nervous system.

BACKGROUND OF THE INVENTION

[0002] Nervous system-related diseases manifest in a variety of forms and seriously endanger people's health and quality of life.

[0003] Stroke is a common acute cerebrovascular disease in middle-aged and elderly people, and tends to attack the younger. It is one of the top three diseases (cancers, cardiovascular diseases and diabetes) harmful to humans in the world today. It is estimated that nearly three million people die from cerebrovascular diseases every year in China. This number is 4 to 5 times higher than that of the US and European countries, 3.5 times higher than that of Japan, and even higher than that of developing countries such as Thailand and India. The incidence rate increases at a rate of 8.7% per year. The recurrence rate exceeds 30%, and the rate of recurrence within five years reaches 54%. 75% of stroke survivors more or less lose their labor capacity and 40% are severely disabled.

[0004] Stroke can be roughly divided into two categories, namely ischemic stroke and hemorrhagic stroke, of which ischemic stroke accounts for 85% of the total number of stroke patients. At present, therapeutic drugs for ischemic stroke mainly includes the following types: vasodilators (such as persantine), drugs that improve microcirculation and expand blood volume (such as low molecular dextran), thrombolytic drugs (such as urokinase), anticoagulant drugs, drugs that prevent platelet aggregation (such as aspirin), Chinese medicine, neuroprotective agents, etc. However, because most of these drugs have issues like significant side effects, potential risks, or insufficient therapeutic efficiency, study on the pathogenesis of stroke and development of drugs directed to the pathogenesis have important social significance for the prevention and treatment of occurrence and development of cerebrovascular diseases.

[0005] Stroke is characterized by neuronal cell death in the regions of local ischemia, cerebral hemorrhage, and/or trauma. Neuron death or injuries caused by cerebral ischemia undergo an injury cascade process, i.e., after occurrence of cerebral ischemia, tissue blood perfusion decreases, excitatory neurotransmitters increase which in turn activates NMDA and AMPA receptors, causes ion channel opening and calcium ion influx, and further activates a large number of enzymes to trigger a signal cascade, resulting in nerve cell damage via multiple pathways. Downstream postsynaptic density 95 protein (PSD-95) triggers a series of ischemic injuries through interaction with various proteins, and therefore is a critical factor for injuries caused by cerebral ischemia, and also a potential target for drug therapy. Therefore, development of PSD-95 inhibitors has great medical significance to nervous system injuries caused by various excitatory neurotoxicity, including stroke.

[0006] In addition, studies have shown that excitatory neurotransmitter NMDA plays an important role in anxiety, epilepsy, and various neurodegenerative diseases such as Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Parkinson's disease, or Huntington's disease. For example, studies have shown that excessive excitation of the central glutamatergic system can cause anxiety, while the NMDA receptor (NMDAR) is a major element responsible for glutamic acid excitatory neurotoxicity. The onset of epilepsy includes three different but continuous pathophysiological processes, including initiation, maintenance and expansion of seizure discharge, and inhibition of seizure discharge. During this process, excitatory neurotransmitters, such as glutamic acid and aspartic acid, play an important role. In Alzheimer's disease, PSD-95 is involved in the neurotoxic mechanism of the disease through the GluR6-PSD-95-MLK3 pathway. Furthermore, in Huntington's disease, PSD-95 is a mediator of neurotoxicity caused by NMDA receptors and huntingtin mutants. Therefore, development of PSD-95 inhibitors is also important for the treatment, amelioration and prevention of the above diseases.

SUMMARY OF THE INVENTION

[0007] In a first aspect, there is provided in the present application a pharmaceutically acceptable salt of a polypeptide, wherein the polypeptide comprises the amino acid sequence YEKLLDTEI (SEQ ID NO: 1) or a functional variant thereof.

[0008] In some embodiments, the functional variant is a variant generated by one or more conservative substitutions in the LDTEI segment of SEQ ID NO: 1.

[0009] In some embodiments, the conservative substitution is selected from the group consisting of a substitution between D and E, a substitution among L, V and I, and a substitution between T and S.

[0010] In some embodiments, the functional variant is a variant generated by replacing the LDTEI segment of SEQ ID NO: 1 with a sequence selected from the group consisting of LDTEL, LDTEV, LDTDI, LDTDLE, LDTDV, LDSEI, LDSEL,

LDSEV, LDSDI, LDSDL, LSDSV, LETEI, LETEL, LETEV, LETDI, LETDL, LETDV, VDTEI, VDTEL, VDTEV, VDTD, VDTD, IDTEI, IDTEL, IDTEV, IDTDI, IDTDL, IDTDV, IETEI, IETEL, IETEV, IETDI, IETDL, and IETDV

[0011] In some embodiments, the polypeptide is a chimeric peptide comprising an internalization peptide moiety and an active peptide moiety, wherein the active peptide moiety is the amino acid sequence YEKLLDTEI (SEQ ID NO: 1) or a functional variant thereof, and the internalization peptide moiety is capable of facilitating uptake of the chimeric peptide by a cell.

[0012] In some embodiments, the internalization peptide moiety comprises the amino acid sequence YGRKKRRQRRR (SEQ ID NO: 2).

[0013] In some embodiments, the chimeric peptide comprises the amino acid sequence YGRKKRRQRRRYEKLLDTEI (SEQ ID NO: 3).

[0014] In some embodiments, the pharmaceutically acceptable salt is selected from the group consisting of a trifluoroacetate, an acetate, a hydrochloride and a phosphate.

[0015] In a second aspect, there is provided in the present application a pharmaceutical composition comprising the pharmaceutically acceptable salt of a polypeptide according to the first aspect, and a pharmaceutically acceptable carrier, excipient, and/or diluent.

[0016] In some embodiments, the pharmaceutical composition is a pre-lyophilized formulation, preferably comprising histidine and trehalose.

[0017] In some embodiments, the pharmaceutical composition is a lyophilized formulation, preferably prepared by lyophilizing the pre-lyophilized formulation as described above.

[0018] In some embodiments, the pharmaceutical composition is a reconstituted formulation, preferably prepared by combining the lyophilized formulation as described above with an aqueous solution.

[0019] In some embodiments, the pharmaceutical composition is for use in the treatment, amelioration or prevention of a nervous system injury, a disease or pain associated with a nervous system injury, a neurodegenerative disease, anxiety or epilepsy in a subject.

[0020] In some embodiments, the pharmaceutical composition is for use as a neuroprotective agent.

[0021] In a third aspect, the present application provides a method for treating, ameliorating or preventing a nervous system injury, a disease or pain associated with a nervous system injury, a neurodegenerative disease, anxiety or epilepsy in a subject, comprising administering to the subject the pharmaceutically acceptable salt of a polypeptide according to the first aspect or the pharmaceutical composition according to the second aspect.

[0022] In a fourth aspect, the present application provides use of the pharmaceutically acceptable salt of a polypeptide according to the first aspect or the pharmaceutical composition according to the second aspect in the preparation of a medicament for the treatment, amelioration or prevention of a nervous system injury, a disease or pain associated with a nervous system injury, a neurodegenerative disease, anxiety or epilepsy in a subject, or in the preparation of a neuroprotective agent.

[0023] In some embodiments of the second, third or fourth aspect, the nervous system injury is a nervous system injury caused by excitatory neurotoxicity.

[0024] In some embodiments, the nervous system injury caused by excitatory neurotoxicity comprises an injury selected from the group consisting of a stroke, a spinal cord injury, an ischemic or traumatic injury to a brain or spinal cord, an injury to a neuron in central nervous system (CNS) including an acute CNS injury, an ischemic stroke or a spinal cord injury, a hypoxia, ischemia, or mechanical injury and an injury caused by a neurodegenerative disease, anxiety, epilepsy or stroke.

[0025] In some embodiments of the second, third, and fourth aspects, the neurodegenerative disease is selected from the group consisting of Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Parkinson's disease, and Huntington's disease.

[0026] In some embodiments of the second, third or fourth aspect, the nervous system injury or pain is located in the peripheral nervous system or the central nervous system.

[0027] In some embodiments of the second, third or fourth aspect, the disease associated with a nervous system injury is a stroke. In some embodiments, the stroke includes an ischemic stroke, a hemorrhagic stroke, and a hemorrhagic stroke converted from an ischemic stroke. In some embodiments, the stroke is an ischemic stroke.

[0028] In some embodiments of the second, third or fourth aspect, the subject is a mammal, such as a non-primate or a primate, e.g. human.

BRIEF DESCRIPTION OF THE DRAWINGS

[0029]

Figure 1 shows the result of a pull-down assay to detect interaction between P5 and PDZ1/2 domain. M represents a protein molecular weight marker; Lane 1 shows His+PDZ1/2+P5; Lane 2 shows P5 alone; Lane 3 shows His+P5;

and Lane 4 shows His+PDZ1/2. The eluted band shown in Lane 1 contains both P5 and PDZ1/2, confirming that P5 is capable of binding to PDZ1/2 domain.

Figure 2 shows a comparison of experimental data of pharmacodynamics of different salts of the polypeptide in rats.

Figure 3 shows the results of cytotoxicity assays for different salts of the polypeptide.

Figure 4 shows the stability of different salts of the polypeptide, in which panels A and B respectively show the content and number of impurity species of different salts of the polypeptide in solid form after exposure to treatments including irradiation+UV, high temperature and high humidity; and panels C and D respectively show the content and the number of impurity species of different salts of polypeptide in the form of aqueous solution after being subjected to irradiation and high temperature treatments.

DETAILED DESCRIPTION OF THE INVENTION

[0030] The inventors of the present application have conducted extensive studies on peptides that reduce the injury effects of neurological disorders mediated at least in part by NMDAR excitatory neurotoxicity. Without wishing to be bound by any particular theory, it is believed that such peptides function, at least in part, by inhibiting the interaction between NMDAR and postsynaptic density 95 protein (PSD-95), i.e., as PSD-95 inhibitors. On the basis of the above, the inventors of the present application have intensively considered various targets for the treatment of nervous system diseases, designed and screened polypeptide neuroprotective agents via *in vivo* and *in vitro* pharmacological and pharmacodynamics experiments, and further improved the obtained peptides to obtain pharmaceutically acceptable salts of the polypeptides with desirable properties.

[0031] Unless otherwise indicated, the terms used in the present application have the meaning as commonly understood by one of ordinary skill in the art.

[0032] The one-letter or three-letter abbreviations for amino acids used in the present application are consistent with international conventions.

[0033] In the specification and claims, the words "including", "comprising" and "containing" mean "including but not limited to" and are not intended to exclude other parts, additives, components or steps.

[0034] In a first aspect, there is provided in the present application a pharmaceutically acceptable salt of a polypeptide, wherein the polypeptide comprises the amino acid sequence YEKLLDTEI (SEQ ID NO: 1) or a functional variant thereof.

[0035] The term "functional variant" refers to a variant having same or similar biological function and property as its parent. As a non-limiting example, a "functional variant" can be obtained by performing one or more conservative substitutions in the parent. The amino acid sequence YEKLLDTEI (SEQ ID NO: 1) or a functional variant thereof is also referred to herein as an "active peptide moiety", which acts as an active moiety in the treatment of a central nervous system injury or in the use as a neuroprotective agent in the present application.

[0036] It has been reported in some studies that some active peptides that inhibit the interaction between NMDAR and PSD-95 are based on the structure of NMDAR. For example, NMDAR2B (GenBank ID 4099612) has 20 amino acids FNGSSNGHVYEKLLSLESDV at its C-terminus including the PL motif ESDV. Some known active peptides contain a part of the amino acid sequence at the C-terminus of NMDAR2B, thereby competitively inhibiting PSD-95 with NMDAR2B. Studies have suggested that the ESDV or LESDV segment in the above peptides plays an important role in inhibiting the interaction between NMDAR and PSD-95. The inventors of the present application obtained a peptide sequence YEKLLDTEI via analysis and validation. As compared with the amino acid composition at the C-terminus of the above NMDAR2B, this peptide lacks two residues SS following KL while having the amino acid sequence YEKL extending from the N-terminus of the PL motif. The inventors of the present application proved that this sequence could enhance the interaction of an active peptide with the PDZ1/2 domain. The LDTEI segment at the C-terminus of the peptide relative to the YEKL motif can be modified, and it is expected that such a modification does not affect the activity of the active peptide or may even increase its activity. Accordingly, in some embodiments, the functional variant provided herein is a variant generated by one or more conservative substitutions in the LDTEI segment of SEQ ID NO: 1.

[0037] In some embodiments, the conservative substitution is selected from the group consisting of a substitution between D and E, a substitution among L, V and I, and a substitution between T and S.

[0038] In some more particular embodiments, the functional variant is a variant generated by replacing the LDTEI segment of SEQ ID NO: 1 with a sequence selected from the group consisting of LDTEL, LDTEV, LDTDI, LDTDV, LDSEI, LDSEL, LDSEV, LDSDI, LDSDL, LDSDV, LETEI, LETEL, LETEV, LETDI, LETDL, LETDV, VDTEI, VDTEL, VDTEV, VDTDI, VDTDV, IDTEI, IDTEL, IDTEV, IDTDI, IDTDL, IDTDV, IETEI, IETEL, IETEV, IETDI, IETDL, and IETDV.

[0039] In some embodiments, the functional variants disclosed herein also comprise an amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or even higher

identity to the peptides as mentioned above. It is known in the art that "identity" between two proteins can be determined by aligning the amino acid sequence of a first protein with the sequence of a second protein which comprises a conservative amino acid substitution relative to the first protein. The degree of identity between two proteins can be determined using computer algorithms and methods well-known to those skilled in the art. The identity between two amino acid sequences is preferably determined by using the BLASTP algorithm.

[0040] In some embodiments, the functional variants disclosed herein include those having substitutions, deletions, additions and/or insertions of amino acid residues at 1, 2, 3, 4, 5 or more positions as compared with the peptides as mentioned above, thereby differing from the particular peptides disclosed above.

[0041] As described above, a functional variant can differ from a particular peptide disclosed above in one or more substitutions, deletions, additions, and/or insertions. Such variants may be naturally occurring or synthetically produced. For example, one or more of the above-described peptide sequences disclosed herein can be modified and their biological activities can be evaluated following any of a variety of techniques well-known in the art or described herein.

[0042] In some embodiments, the polypeptide is a chimeric peptide comprising an internalization peptide moiety and an active peptide moiety, wherein the active peptide moiety is the amino acid sequence YEKLLDTEI (SEQ ID NO: 1) or a functional variant thereof, and the internalization peptide moiety is capable of facilitating uptake of the chimeric peptide by a cell.

[0043] It should be understood by those skilled in the art that the main purpose of combining an active peptide and an internalization peptide into a chimeric peptide is to better deliver the active peptide to the target of action. Therefore, internalization peptides suitable for the present application are not limited to specific types, as long as the cell-penetrating purpose can be achieved. It should also be understood by those skilled in the art that since the target of action of the active peptide is mainly located inside neuronal cells, it is preferred that the internalization peptide is specifically appropriate to neuronal cells. In some embodiments, the internalization peptide can be a Tat peptide. In some embodiments, the amino acid sequence of a Tat peptide is YGRKKRRQRRR (SEQ ID NO: 2). In some embodiments, the chimeric peptide comprises the amino acid sequence YGRKKRRQRRRYEKLLDTEI (SEQ ID NO: 3).

[0044] It should be appreciated that an internalization peptide may be linked to an active peptide via an amide bond to form a fusion peptide, while they may also be linked via other suitable means, such as chemical bonding. Coupling of two components can be achieved with a coupling agent or a conjugating agent. A great number of such reagents are commercially available and can be found in S. S. Wong, Chemistry of Protein Conjugation and Cross-Linking, CRC Press (1991). Examples of cross-linking reagents include J-succinimide-3-(2-pyridinedithio)propionate (SPOP) or N,N'-(1,3-phenylene) bismaleimide; N, N'-ethylidene-bis-(iodoacetamide) or other such reagents having 6 to 11 carbon methylene bridges (which are relatively specific to thiol groups); and 1,5-difluoro-2,4-dinitrobenzene (which forms an irreversible linkage with an amino group and an tyrosine group). Other cross-linking reagents include P,P'-difluoro-m,m'-dinitrodiphenyl sulfone (which forms an irreversible cross-linkage with an amino group and an phenol group); dimethyl diethylamine hexanoate (which is specific to an amino group); phenol-1,4-disulfonyl chloride (which mainly reacts with an amino group); 1,6-hexamethylene diisocyanate or diisothiocyanate, or phenylazo-p-diisocyanate (which mainly reacts with an amino group); glutaraldehyde (which reacts with several different side chains) and bis-diazotized benzidine (which mainly reacts with tyrosine and histidine).

[0045] The active peptide and the fusion peptide with the active peptide fused to an internalization peptide of the present application can be synthesized by solid phase synthesis methods or recombinant methods. Peptidomimetics can be synthesized using a variety of protocols and methods described in scientific literatures and patent literatures, such as Organic Syntheses Collective Volumes, Gilman et al. (ed.) John Wiley & Sons, Inc., NY, al-Obeidi (1998) Mol. Biotechnol. 9:205-223; Hruby (1997) Curr. Opin. Chem. Biol. 1: 114-119; Ostergaard (1997) Mol. Divers. 3: 17-27; Ostresh (1996) Methods Enzymol. 267: 220-234.

[0046] Without wishing to be bound by any particular theory, it is expected that a molecule or ion with charge opposite to a drug, when combining with the drug to form a salt, can improve certain undesirable physicochemical or biopharmaceutical properties of the drug, such as changing solubility or dissolution, reducing hygroscopicity, improving stability, or changing melting point for the drug. The final determination of an ideal salt form requires proper balance between physicochemical properties and biopharmaceutical properties. Requirements, e.g. solubility, hygroscopicity, and stability to environmental factors in different states, should be given priority in selecting a pharmaceutically acceptable salt form of a drug. The pharmaceutically acceptable salts of the polypeptides of the present application may be in any suitable pharmaceutically acceptable salt form. In some embodiments, the pharmaceutically acceptable salt of the polypeptide is a trifluoroacetate. In some embodiments, the pharmaceutically acceptable salt of the polypeptide is an acetate. In some embodiments, the pharmaceutically acceptable salt of the polypeptide is a hydrochloride. In some embodiments, the pharmaceutically acceptable salt of the polypeptide is a phosphate. In some particular embodiments, the pharmaceutically acceptable salt of the polypeptide is an acetate or a hydrochloride.

[0047] In a second aspect, there is provided in the present application a pharmaceutical composition comprising the pharmaceutically acceptable salt of a polypeptide as described in the first aspect, and a pharmaceutically acceptable carrier, excipient, and/or diluent.

[0048] The compounds described herein can be prepared in the form of a lyophilized formulation. In some embodiments, the present application provides a lyophilized formulation. The lyophilized formulation can be prepared from a pre-lyophilized formulation by lyophilization, which contains at least an active ingredient, a buffer, a filler and water, wherein the active ingredient is the compound of the present application or a pharmaceutically acceptable salt thereof. In some

embodiments, a preferred buffer is histidine. Other buffers can be selected from succinate, citrate, gluconate, acetate, phosphate, Tris, etc. The filler provides a structure to the lyophilized compound. In some embodiments, the filler is selected from mannitol, trehalose, dextran-40, glycine, lactose, sorbitol, sucrose, and the like, with trehalose being preferred. In some embodiments, the lyophilized formulation of the present application comprises a compound described above, or a pharmaceutically acceptable salt thereof, and histidine and trehalose.

[0049] The lyophilized formulation can be reconstituted. That is, the lyophilized formulation is rehydrated with a solution to form a solution of particles that are invisible to naked eyes. In some embodiments, the present application provides a reconstituted formulation prepared by combining a lyophilized formulation with an aqueous solution. In some embodiments, the aqueous solution is water for injection. In some embodiments, the aqueous solution is a physiological saline.

[0050] The term "lyophilization" relates to a process in which the raw materials to be dried are first frozen and then sublimed in a vacuum environment to remove ice or frozen solvents.

[0051] In some embodiments, a pharmaceutically acceptable salt of a polypeptide disclosed herein can be administered in the form of a pharmaceutical composition. The pharmaceutical composition can be prepared by conventional methods, e.g., mixing, dissolving, granulating, tableting, milling, emulsifying, encapsulating, capturing or lyophilizing.

[0052] The pharmaceutical composition can be formulated in a conventional manner using one or more physiologically acceptable carriers, diluents, excipients or ingredients suitable for preparing a pharmaceutically acceptable salt of a polypeptide into a pharmaceutically acceptable formulation. Proper formulation depends on chosen administration routes.

[0053] In some embodiments, the administration can be parenteral, intravenous, oral, subcutaneous, intraarterial, intracranial, intrathecal, intraperitoneal, topical, intranasal, or intramuscular administration. Intravenous administration is preferred.

[0054] In some embodiments, the pharmaceutical composition for parenteral administration is preferably sterile and substantially isotonic. For injection, a pharmaceutically acceptable salt of a polypeptide can be formulated in an aqueous solution, preferably in a physiologically compatible buffer such as Hank's solution, Ringer's solution, or physiological saline or acetate buffer (to reduce discomfortableness at the injection site). The solution may contain formulating agents such as suspending, stabilizing and/or dispersing agents.

[0055] Alternatively, a pharmaceutically acceptable salt of a polypeptide may be in the form of a powder for re-constitution with a suitable vehicle, such as sterile non-pyrogenic water, prior to use.

[0056] For transmucosal administration, penetrants appropriate for penetrating the barrier of interest are used in the formulation. This administration route can be used to deliver a compound to the nasal cavity or for sublingual administration.

[0057] In some embodiments, for oral administration, a pharmaceutically acceptable salt of a polypeptide can be formulated with a pharmaceutically acceptable carrier into tablets, pills, troches, capsules, liquids, gels, syrups, slurries, suspensions or the like, for oral ingestion by a patient to be treated. For oral solid formulations such as powders, capsules and tablets, suitable excipients include fillers such as sugars such as lactose, sucrose, mannitol and sorbitol; cellulose preparations such as corn starch, wheat starch, rice starch, potato starch, gelatin, tragacanth, methylcellulose, carboxypropylmethylcellulose, sodium carboxymethylcellulose and/or povidone (PVP); granulating agents and binders. If required, a disintegrating agent, such as crosslinked polyvinylpyrrolidone, agar, or alginic acid or a salt thereof (such as sodium alginate), may be added. If required, solid formulations can be coated with sugar or enteric coating using standard techniques. For oral liquid formulations such as suspensions, elixirs and solutions, suitable carriers, excipients or diluents include water, glycerol, oil and alcohol. Furthermore, a flavoring agent, a preservative, a coloring agent, or the like may be added.

[0058] In addition to the formulations as described above, a pharmaceutically acceptable salt of a polypeptide can be formulated into a reservoir preparation. Such long-acting formulations can be administered by implantation (for example subcutaneous or intramuscular) or by intramuscular injection. Thus, for example, a compound can be formulated with a suitable polymeric or hydrophobic material (for example, formulated as an emulsion in an acceptable oil) or an ion exchange resin, or formulated as a sparingly soluble derivative, for example, a sparingly soluble salt.

[0059] Alternatively, other drug delivery systems can be used. A chimeric peptide can be delivered using liposomes and emulsions. Certain organic solvents, such as dimethyl sulfoxide, can also be used. Additionally, a compound can be delivered using a sustained release system, such as a semipermeable substrate of solid polymers containing a therapeutic agent.

[0060] Sustained release capsules may release a chimeric peptide for several weeks or even up to over 100 days depending on their chemical properties. Other strategies for stabilizing a protein can be used depending on the chemical property and biostability of a therapeutic agent.

[0061] A pharmaceutically acceptable salt of a polypeptide is used in an amount effective to achieve an intended

purpose (e.g., to ameliorate the damaging effect of stroke injuries and related conditions). A therapeutically effective amount means an amount of a pharmaceutically acceptable salt of a polypeptide sufficient to significantly reduce the injuries caused by stroke in patients (or a model animal population) treated with a pharmaceutically acceptable salt of a polypeptide disclosed herein, as compared with the central nervous system injury in a control population of patients (or model animals) not treated with a pharmaceutically acceptable salt of a polypeptide disclosed herein. If a treated patient achieves a better output as compared with a mean output (as determined by infarction volume or disability index) in a comparable patient control population not treated by a method disclosed herein, the amount is also considered to be therapeutically effective. The amount is also considered to be a therapeutically effective amount. If a treated patient shows 2 or fewer disability scores in the Rankin scale and 75 or more scores in the Barthel scale, the amount is also considered to be a therapeutically effective amount. If a treated patient population shows a significantly improved (i.e., less disability) score distribution in the disability scale as compared with comparable untreated populations, the dose is also considered to be therapeutically effective, see Lees et al. N Engl J Med 2006; 354: 588-600. A therapeutically effective regimen represents a combination of a therapeutically effective dose and an administration frequency required to achieve the above intended purpose.

[0062] In some embodiments, a preferred dose range of a pharmaceutically acceptable salt of a polypeptide of the present application comprises 0.001 to 20 μmol of the salt per kg patient body weight, optionally 0.03 to 3 μmol of the salt per kg patient body weight, including any value or any range therebetween. In some methods, 0.1-20 μmol of a salt of the present application per kg patient body weight is administered within 6 hours. In some methods, 0.1-10 μmol of a salt of the present application per kg patient body weight is administered within 6 hours, more preferably about 0.3 μmol of a salt of the present application per kg patient body weight is administered within 6 hours. In other instances, the dose range is 0.005 to 0.5 μmol of a salt of the present application per kg patient body weight. The dose per kg body weight can be converted from rats to humans by dividing by 6.2 to compensate for different surface area: mass ratios. In gram, suitable dose of a salt of the present application for human use may include 0.01 to 100 mg/kg patient body weight, or more preferably 0.01 to 30 mg/kg patient body weight or 0.01 to 10 mg/kg, or 0.01 to 1 mg/kg, including any value or any range therebetween.

[0063] In some embodiments, the administered amount of a pharmaceutically acceptable salt of a polypeptide depends on the subject being treated, the weight of the subject, the pain severity, the administration mode, and the adjustments by the prescribing physician. Therapy can be repeated when symptoms are detectable or even undetectable. Therapy can be provided alone or in combination with other drugs.

[0064] In some embodiments, a therapeutically effective dose of a pharmaceutically acceptable salt of a polypeptide disclosed herein is capable of providing a therapeutic benefit without causing significant toxicity. The toxicity of a chimeric peptide can be determined in cell cultures or experimental animals by standard pharmaceutical procedures, for example by determining LD_{50} (a dose that kills 50% of the population) or LD_{100} (a dose that kills 100% of the population). The dose ratio between toxic effect and therapeutic effect is a therapeutic index. A pharmaceutically acceptable salt of a polypeptide exhibiting a high therapeutic index is preferred (see, for example, Fingl et al, 1975, In: The Pharmacological Basis of Therapeutics, Chapter 1, page 1).

[0065] In a second aspect, there is provided in the present application a pharmaceutical composition comprising the pharmaceutically acceptable salt of a polypeptide as described in the first aspect, and a pharmaceutically acceptable carrier, excipient, and/or diluent.

[0066] In some embodiments, the pharmaceutical composition is used for treating, ameliorating or preventing a nervous system injury, a disease or pain associated with a nervous system injury, a neurodegenerative disease, anxiety or epilepsy in a subject.

[0067] In some embodiments, the pharmaceutical composition is used as a neuroprotective agent.

[0068] In some embodiments, the nervous system injury is a nervous system injury caused by excitatory neurotoxicity.

[0069] In some embodiments, the nervous system injury caused by excitatory neurotoxicity comprises an injury selected from the group consisting of a stroke, a spinal cord injury, an ischemic or traumatic injury to a brain or spinal cord, an injury to a neuron in central nervous system (CNS) including an acute CNS injury, an ischemic stroke or a spinal cord injury, a hypoxia, ischemia, or mechanical injury and an injury caused by a neurodegenerative disease, anxiety, epilepsy or stroke.

[0070] In some embodiments, the pharmaceutical composition is used for treating, ameliorating or preventing an ischemic stroke or a nervous system injury caused by an ischemic stroke. In some embodiments, the pharmaceutical composition is used for treating, ameliorating or preventing a hemorrhagic stroke or a nervous system injury caused by a hemorrhagic stroke. In some embodiments, the pharmaceutical composition is used for treating, ameliorating or preventing a hemorrhagic stroke converted from an ischemic stroke or a nervous system injury caused by a hemorrhagic stroke converted from an ischemic stroke.

[0071] Stroke is a condition caused by impaired blood flow in the CNS. Possible causes include embolism, bleeding, and thrombosis. Some neuronal cells die immediately due to impaired blood flow. These cells release their component molecules (including glutamic acid), which in turn activate NMDA receptors, which increase intracellular calcium levels

and intracellular enzyme levels, resulting in death of more neuronal cells (excitatory neurotoxicity cascade amplification). The death of CNS tissues is called as infarction. An infarction volume (i.e., the volume of dead neuronal cells in the brain caused by stroke) can be used as an indicator of the extent of pathological injuries caused by stroke. Symptomatic effects depend on both the infarction volume and the location of the infarction in the brain. A disability index can be used as a measure of symptomatic injuries, such as the Rankin Stroke Outcome Scale (Rankin, Scott MedJ; 2: 200-15 (1957) and the Barthel Index. The Rankin Scale is based on a direct assessment of a patient's systemic condition as follows.

0: completely no symptom.

1: with symptoms, but no significant disability; able to perform all daily work and activities.

2: minor disability; unable to perform all previous activities, but able to take care of their own affairs without help.

3: moderate disability that requires some help, but able to walk without help.

4: moderate to severe disability, unable to walk without help, and unable to take care of their own body requirements without help.

5: severe disability; bedridden, incontinence, and requiring lasting care and attention.

[0072] The Barthel Index is based on a series of questions about the patient's ability to perform 10 basic daily living activities, which are scored between 0 and 100, with lower scores indicating more disability (Mahoney et al., Maryland State Medical Journal) 14:56-61 (1965).

[0073] Alternatively, stroke severity/output can be measured using the NIH Stroke Scale, which is available on the World Wide Web at ninds.nih.gov/doctors/NIH_Stroke_Scale_Booklet.pdf. The Scale is based on a patient's ability to perform 11 sets of functions, including assessment of a patient's consciousness, movement, feeling, and language function levels.

[0074] An ischemic stroke more clearly specifies a type of stroke caused by blockage of blood flow to the brain. The potential pathology for such blockages is most commonly associated with the occurrence of fat deposits along the walls of blood vessels. This condition is called as atherosclerosis. These fat deposits can cause two types of obstruction. Cerebral thrombosis refers to a thrombus (blood clot) formed in a blocked part of a blood vessel. "Brain embolism" usually means that various emboli in the blood (such as a wall thrombus in the heart, atherosclerotic plaque, fat, tumor cells, fibrocartilage or air) enter the cerebral artery along with blood flow to block blood vessels. When the collateral circulation is not sufficient for compensation, it causes ischemic necrosis of brain tissue to which the artery supplies blood, and focal neurologic impairment. The second important cause of embolism is irregular heartbeats called arterial fibrillation. This causes a condition that a blood clot can be formed in the heart, and then moves and transfers to the brain. Other potential causes of an ischemic stroke are hemorrhage, thrombosis, arterial or venous severing, cardiac arrest, shock from any causes (including bleeding), and iatrogenic causes, such as direct surgical injuries to cerebral blood vessels or blood vessels going to the brain or cardiac surgery. Ischemic strokes account for approximately 83% of all stroke cases.

[0075] Several other neurological disorders can also cause neuron death through NDMAR-mediated excitatory neurotoxicity. These disorders include neurodegenerative diseases, anxiety, epilepsy, hypoxia, damage to the CNS irrelevant to a stroke, such as traumatic brain injury and spinal cord injury. Accordingly, in some embodiments, the pharmaceutical composition is used for treating, ameliorating or preventing neurodegenerative diseases, anxiety or epilepsy, wherein the neurodegenerative diseases may comprise Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Parkinson's disease or Huntington's disease.

[0076] In some embodiments, the pharmaceutical composition is used as a neuroprotective agent.

[0077] In a third aspect, there is provided in the present application a method for treating, ameliorating or preventing a nervous system injury, a disease or pain associated with a nervous system injury, a neurodegenerative disease, anxiety or epilepsy in a subject, comprising administering to the subject the pharmaceutically acceptable salt of a polypeptide as described in the first aspect or a pharmaceutical composition as described in the second aspect.

[0078] In some embodiments, the nervous system injury is a nervous system injury caused by excitatory neurotoxicity, wherein the injury or pain may be located in the peripheral nervous system or the central nervous system. In some embodiments, the nervous system injury caused by excitatory neurotoxicity comprises an injury selected from the group consisting of a stroke, a spinal cord injury, an ischemic or traumatic injury to a brain or spinal cord, an injury to a neuron in central nervous system (CNS) including an acute CNS injury, an ischemic stroke or a spinal cord injury, a hypoxia, ischemia, or mechanical injury and an injury caused by a neurodegenerative disease, anxiety, epilepsy or stroke.

[0079] In some embodiments, the neurodegenerative disease includes Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Parkinson's disease, or Huntington's disease.

[0080] In some embodiments, the disease is an ischemic stroke or a nervous system injury caused by an ischemic stroke. In some embodiments, the disease is a hemorrhagic stroke or a nervous system injury caused by a hemorrhagic stroke. In some embodiments, the disease is a hemorrhagic stroke converted from an ischemic stroke or a nervous system injury caused by a hemorrhagic stroke converted from an ischemic stroke.

[0081] In a fourth aspect, there is provided in the present application use of the pharmaceutically acceptable salt of a polypeptide as described in the first aspect, or a pharmaceutical composition as described in the second aspect, in the preparation of a medicament for treating, ameliorating or preventing a nervous system injury, a disease or pain associated with a nervous system injury, a neurodegenerative disease, anxiety or epilepsy in an subject, or in the preparation of a neuroprotective agent.

[0082] In some embodiments, the nervous system injury is a nervous system injury caused by excitatory neurotoxicity, wherein the injury or pain may be located in the peripheral nervous system or the central nervous system. In some embodiments, the nervous system injury caused by excitatory neurotoxicity comprises an injury selected from the group consisting of a stroke, a spinal cord injury, an ischemic or traumatic injury to a brain or spinal cord, an injury to a neuron in central nervous system (CNS) including an acute CNS injury, an ischemic stroke or a spinal cord injury, a hypoxia, ischemia, or mechanical injury and an injury caused by a neurodegenerative disease, anxiety, epilepsy or stroke.

[0083] In some embodiments, the neurodegenerative disease includes Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Parkinson's disease, or Huntington's disease.

[0084] In some embodiments, the disease is an ischemic stroke or a nervous system injury caused by an ischemic stroke. In some embodiments, the disease is a hemorrhagic stroke or a nervous system injury caused by a hemorrhagic stroke. In some embodiments, the disease is a hemorrhagic stroke converted from an ischemic stroke or a nervous system injury caused by a hemorrhagic stroke converted from an ischemic stroke.

[0085] The term "subject", as used herein, refers to animals including birds, reptiles, and mammals. In some embodiments, the subject is a mammal, including primates and non-primates, such as human, chimpanzees, bovines, equines, pigs, sheep, goats, dogs, cats, and rodent animals such as rats and mice.

[0086] It should be understood that the foregoing detailed description only aims to help those skilled in the art to more clearly understand the present application, but not intended to limit the present application in any way. Those skilled in the art can make various modifications and changes to the described embodiments.

EXAMPLES

[0087] The following examples are provided only to illustrate some embodiments of the present application without any purpose or nature of limitation.

Example 1: Screening of active peptide molecules

[0088] The Tat transmembrane peptide YGRKKRRQRRR (SEQ ID NO: 2) was selected based on reported study results, and ligated to various numbers of amino acids to form a peptide library. The chimeric peptide molecules in the peptide library were tested for interaction with the PDZ1/2 domain expressed and purified *in vitro*, and the polypeptides were preliminarily screened for the strength of interaction force.

[0089] The immobile phase molecule (ligand) was PDZ1/2 protein with a molecular weight of approximately 20kD at a concentration of 2mg/ml. The mobile phase molecule (analyte) was a polypeptide to be screened with a molecular weight of approximately 2 kD at a concentration of 10mg/ml. The CM5 chip was used for fixation using a Biacore 3000 instrument. The electrophoresis buffer was PBS plus 0.005% Tween 20. Fixation was carried out using an amino coupling method. The concentration of the ligand was 10 µg/ml. The fixation buffer was 10 mM sodium acetate, pH 4.0. The fixed amount was 1400 RU, which was fixed to flow cell 2. The used flow rate was 10 µg/ml and the ligand was loaded for 1 minute. 10 mM Gly at pH 2.0 + 2.5 was used as a regenerant. Regeneration was carried out at a flow rate of 30 µl/min. The loading time was 30 s.

[0090] Kinetic analysis was performed using the following conditions.

Control Channel: flow cell 1

Electrophoresis Buffer: PBS

Mode: Kinetic Analysis Wizard

Concentration Gradients: 6.25nM, 12.5nM, 25nM, 50nM, 100nM, 200nM, 400nM

Loading Time: 1 minute

Dissociation Time: 2 min

Flow Rate: 30 µl/min

[0091] The data was fitted using the fitting software Biaevaluation 4.1. The fitting model was a 1:1 binding model. The dissociation constant KD value was inversely proportional to the interaction force.

[0092] By screening, a chimeric peptide having strong capability of interacting with the PDZ1/2 domain was obtained, and named as P5. The sequence of the chimeric peptide was shown below.

P5: YGRKKRRQRRRYEKLLDTEI

[0093] In order to directly compare with a similar chimeric peptide in the reported studies, a control chimeric peptide NA-1 was introduced with the following sequence.

NA-1: YGRKKRRQRRRLSSIESDV (SEQ ID NO: 4)

[0094] Furthermore, in view of the structural difference between P5 and NA-1, a chimeric peptide YE-NA-1 having two residues of YE added to the N-terminus of the active peptide of the chimeric peptide NA-1 was additionally introduced, and its sequence is shown below.

YE-NA-1: YGRKKRRQRRRYEKLSSIESDV (SEQ ID NO: 5)

[0095] The chimeric peptides NA-1, YE-NA-1 and P5 were simultaneously subjected to tests for interaction with the PDZ1/2 domain as mentioned above, and the results were shown in Table 1 below.

Table 1. Determination of interaction force between three chimeric peptides and PDZ1/2 domain

chimeric peptides	NA-1	YE-NA-1	P5
KD (M)	7.53E-08	5.44 E-08	2.99E-08

[0096] As shown in Table 1, the chimeric peptides YE-NA-1 and P5 interacted more strongly with the PDZ1/2 domain as compared with the control chimeric peptide NA-1, and the performance of P5 was even better. Therefore, based on the inventors' speculation, the additional two amino acid residues YE at the N-terminus of the active peptide caused certain improved effect on the interaction of the polypeptide with the PDZ1/2 domain. Furthermore, P5 lacked two weakly hydrophobic serines (SS) relative to the carboxy terminus of YE-NA-1. Based on the inventors' speculation, this may further increase the interaction of the polypeptide with the PDZ1/2 domain.

Example 2: Pull-down assay to verify the interaction of P5 with PDZ1/2 domain

[0097] To confirm that P5 can interact with the PDZ1/2 domain, a pull-down assay was performed.

[0098] The column was equilibrated with 100 μ l of His beads and 1 ml of MCAC-0 buffer for 5 min and then shaken at 4 °C. The mixture was centrifuged at 5,000 g for 1 minute at 4 °C, and the supernatant was discarded. 1 mg of PDZ1/2 protein was added to the mixture, and a buffer was added to reach the volume of 1 ml. The mixture was spun for binding for 1 hour at 4 °C. The mixture was centrifuged at 5000 g for 1 minute at 4 °C, and the supernatant was discarded. The mixture was washed three times with 1 ml of MCAC-0 buffer with each time for 5 minutes (at 4 °C, washing with shaking). 1 mg of P5 protein was added to the mixture, and a buffer was added to reach the volume of 1 ml. The mixture was spun for binding for 2 hours at 4 °C. The mixture was centrifuged at 5000 g for 1 minute at 4 °C, and the supernatant was discarded. The mixture was washed three times with 1 ml of lysis buffer with each time for 5 minutes (at 4 °C, washing with shaking). 20 μ l of MCAC-300 was added after washing. After centrifugation, the eluate was taken for an SDS-PAGE assay. The experimental results were shown in Figure 1.

[0099] As shown in Figure 1, both P5 and the PDZ1/2 domain were contained in the eluted band of the chimeric peptide P5, thereby confirming that the chimeric peptide P5 can bind to the PDZ1/2 domain.

Example 3: Therapeutic effects of P5 salts on a rat MCAO model

[0100] Based on the chimeric peptide P5 obtained in Examples 1 and 2, the inventors of the present application designed P5-trifluoroacetate (P5-TFA), P5-acetate (P5-Ac), and P5-hydrochloride (P5-C1), and commissioned Hangzhou Chinese Peptide Co., Ltd. for synthesis. The three prepared P5 salts were tested on a rat MCAO model for their therapeutic effects.

Experimental animals and materials

[0101] The animals were male adult SD rats (Vittalia) of SPF grade with body weight of 220-250 g.

[0102] The instruments included one line scissor, two eye surgery scissors, four curved forceps, 4#, 5# surgical sutures, 6 $\times$ 17 triangular needles, a occlusion line (0.26 mm of diameter), and one needle holders. The agents included Enbipu sodium chloride injection solution (Shijiazhuang Group NBP Pharmaceutical Co., Ltd.), chloral hydrate, furosemide (20mg/vial), gentamicin sulfate (80mg/vial), cotton swabs, and medical trays.

MCAO modeling

[0103] The focal cerebral ischemia-reperfusion model was prepared according to the reversible middle cerebral artery occlusion (MCAO) suture method proposed by Longa with modifications in view of the anatomical structure of the rat brain. The rats were anesthetized by intraperitoneal administration of 10% chloral hydrate at a dose of 0.3ml/kg. After anesthetization, a cut was created at the cervical midline, and the common carotid artery (CCA), external carotid artery

(ECA) and pterygopalatine artery were exposed. The head portion (0.5cm) of a monofilament nylon fishing line (0.26mm) was coated with paraffin and a mark was made at 20mm. All rats were inserted through the right CCA incision, and the pterygopalatine artery was temporarily clamped to prevent mis-insertion. The length of the occlusion line was about 18-20mm from the bifurcation of CCA depending on the animal's weight, thereby occluding middle cerebral artery on the right side. The skin was then sewed, and the tail end of the occlusion line was partially fixed to the skin. After a period of ischemia for 2 hours, the occlusion line was carefully pulled out to form a reperfusion. The body temperature was maintained at 37 ± 0.5 °C during the ischemia period and 2 h after reperfusion. The success marker for the model is that the rats, after they awoke from anesthesia, showed paralyzed left limb, unstable standing and turning to one side when their tails were lift up.

Experimental grouping

[0104] The experimental animals were divided into the control group (normal group), model group (normal saline group), positive control drug Enbipu group (NBP), and P5 salt groups. A saline solution, positive control drug Enbipu (2.5 mg/kg), and each P5 salt (10 mg/kg) were respectively administered to respective groups of rats via tail vein injection at 1 hour after ischemia.

Calculation of infarction volume

[0105] The rats were sacrificed by decapitation at 24h after administration. The brain tissues were quickly removed and placed in a refrigerator at -20 °C. After 10 minutes, the tissues were placed in a room temperature environment. The brains were placed in a rat brain section mold. After the olfactory bulb, cerebellum and low brain stem were removed, the brains were coronally cut five times at 2mm thickness as shown in the profile to obtain six continuous raw coronal slices. Then, the brain sections were quickly placed in a 5 ml solution containing 2% TTC, and incubated at 37 °C for 30 minutes in the dark, during which the brain sections were flipped once every 5 minutes. With the TTC staining, the normal tissue would be rose red, and the infarcted tissue would be unstained and retained white. Each group of brain sections was arranged neatly, and photographed. The photos were processed by an image analysis system software and statistically analyzed. The infarction area of each brain section was calculated, and multiplied by the thickness of each brain section (2 mm). The products of the infarction area of subject brain section multiplied by the thickness were summed to obtain the cerebral infarction volume for each animal. The volumes were expressed as percentages accounting for the cerebral hemisphere to eliminate the effects of cerebral edema.

[0106] The experimental results were shown in Figure 2. The results showed that the administration of P5-TFA, P5-Ac and P5-C1 significantly reduced the cerebral infarction volume in the rats as compared with the model group ($p < 0.01$); while as compared with the positive control drug NBP, the effects of administration of P5-TFA, P5-Ac and P5-C1 in reducing the cerebral infarction volume in the rats were significantly superior to that of the positive control drug NBP ($p < 0.05$).

Example 4: Determination of cytotoxicity of P5 salts

[0107] This example tested the cytotoxicity of two polypeptide salts, P5-Ac and P5-C1 in Example 3.

Experimental materials

[0108] Cell line PC12 cells were used. Other experimental materials included a clean bench with vertical laminar flow, a steam sterilizer, a centrifuge, a microscope, a microplate reader, cover slips, blood cell counting plates, a manual counter, an alcohol lamp, pipettors, pipettes, pipette tips, centrifuge tubes, 96-well plates, PBS/saline, high glucose DMEM medium (containing 10% FBS and 1% dual-antibiotics). CCK8 was purchased from Solvay.

Experimental protocols

[0109] Cells at logarithmic phase were digested and seeded into a 96-well plate at a density of 4×10^4 cells/well in triplicates, and cultured overnight. The P5 salt (e.g., 5 μ M) was formulated in the culture medium. The supernatant in the 96-well plate was discarded, the plate was washed 2-3 times with PBS/physiological saline, and then 100 μ l of P5 salt preparation was added to each well. Same P5 salt preparation was also added to the blank control wells (no cell). The cells were cultured for 24 hours. 10 μ l of CCK8 was added to each well of the 96-well plate and the plate was incubated for 1 h. The absorbance of each well of the 96-well plate was measured at 450 nm using a microplate reader. Cell viability was calculated from the reads based on the following equation.

$$\text{Cell viability (\%)} = (\text{experimental group} - \text{corresponding blank control group}) / (0 \mu\text{M experimental group} - 0 \mu\text{M blank control group}) \times 100\%$$

[0110] The experimental results were shown in Figure 3. The results showed that the two pharmaceutically acceptable salts of the P5 polypeptide, i.e. P5-Ac and P5-C1, had no significant cytotoxicity at a concentration of 5 μM .

Example 5: Stability of P5 salts

[0111] In this example, the stability of the three P5 salts in Example 3 was tested under various conditions including irradiation, high temperature, and high humidity.

Stability analysis of P5 salt powders

[0112] The three P5 salts in Example 3 in their powder form were subjected to treatments with irradiation (3000 Lx) + ultraviolet (UV), high temperature (60° C), and high humidity (75% RH) for 10 days. After the treatments, the powders were dissolved in water to prepare a solution at a concentration of 2mg/ml. 10 μl of each solution was accurately measured and injected into a liquid chromatograph, and the chromatograms were recorded. Relevant substances were calculated according to the area normalization method to analyze the content and the number of impurity species.

Instruments and reagents

[0113] high performance liquid chromatograph (Agilent, 1260 EZChrom); chromatographic column (Agilent, ZORBAX 300SB-C18 (4.6 * 250mm, 5 μm) SN: USHH008416); analytical balance (Sartoris, BT25S); filter membrane (Millipore, 0.45 μm PTFE); acetonitrile (MREDA); water (Aqua); TFA (MREDA); and comprehensive drug stability test box (three-box type) (Shanghai Zuocheng Experimental Instrument Co. Ltd, item no. SHH-3SDT).

Chromatographic parameters

[0114] Mobile phase: A 0.065% TFA-water; B 0.05% TFA-ACN
 Detection wavelength λ = 220nm; flow rate V = 1.0ml/min; temperature T = 36° C
 Injection volume Inj = 10 μl
 Gradient conditions: 0-30min, B% = 5-65%

Result analysis

[0115] The results of the stability analysis of the three P5 salt powders were shown in Table 2 below and panels A and B in Figure 4.

Table 2

		Hydrochloride	Acetate	Trifluoroacetate
Irradiation + ultraviolet	Percentage of main peak area	90.59%	96.47%	98.34%
	Number of impurity species	18	14	6
High temperature	Percentage of main peak area	99.50%	99.79%	99.56%
	Number of impurity species	3	1	3
High humidity	Percentage of main peak area	99.44%	95.75%	99.73%
	Number of impurity species	3	2	1

Results

[0116] After the treatment with irradiation + ultraviolet, the percentages of main peak area and the numbers of impurity species of the three salts were significantly different. The relative stability order was: trifluoroacetate> acetate> hydrochloride.

[0117] After the treatment with high temperature, the percentages of the main peak area of the three salts were all above 99.5% without significant difference. Acetate was the most stable in the comparison of the number of impurity species.

[0118] After the treatment with high humidity, the percentage of the main peak area of the acetate was significantly reduced. The relative stability order was: trifluoroacetate> hydrochloride> acetate.

[0119] In general, the three salts showed good stability under different conditions.

Stability analysis of P5 salt solutions

[0120] The three P5 salts in Example 3 were dissolved in water to prepare solutions at a concentration of 2 mg/ml. The solutions were subjected to treatments with irradiation (3000Lx) and high temperature (60 °C) for 10 days. After the treatments, 10 μ l of each solution was accurately measured and injected into a liquid chromatograph, and the chromatograms were recorded. The relevant substances were calculated according to the area normalization method to analyze the content and the number of impurity species.

Instruments and reagents

[0121] high performance liquid chromatograph (Agilent, 1260 EZChrom); chromatographic column (Agilent, ZORBAX 300SB-C18 (4.6 * 250mm, 5 μ m) SN: USHH008416); analytical balance (Sartoris, BT25S); filter membrane (Millipore, 0.45 μ m PTFE); acetonitrile (MREDA); water (Aqua); TFA (MREDA); and comprehensive drug stability test box (three-box type) (Shanghai Zuocheng Experimental Instrument Co. Ltd, item no. SHH-3SDT).

Chromatographic conditions

[0122] Mobile phase: A 0.065% TFA-water; B 0.05% TFA-ACN
Detection wavelength λ = 220nm; flow rate V = 1.0ml/min; temperature T = 36° C
Injection volume Inj = 10 μ l
Gradient conditions: 0-30min, B% = 5-65%

Result analysis

[0123] The results of the stability analysis of the aqueous solutions of the three P5 salts were shown in Table 3 and panels C and D in Figure 4.

Table 3

		Hydrochloride	Acetate	Trifluoroacetate
Irradiation	Percentage of main peak area	96.73%	98.51%	97.28%
	Number of impurity species	7	4	10
High temperature	Percentage of main peak area	58.63%	90.07%	
	Number of impurity species	11	7	

Results

[0124] After the treatment with irradiation, the percentages of main peak area and the numbers of impurity species of the three salt solutions were different. The relative stability order was: acetate> trifluoroacetate> hydrochloride.

[0125] After the treatment with high temperature, the percentages of main peak area and the numbers of impurity species of the hydrochloride and acetate were different. The relative stability order was: acetate> hydrochloride.

[0126] In general, the three salt solutions also showed good stability under different conditions.

[0127] All publications and patent documents cited in the Specification are herein incorporated by reference as if each publication or patent were specifically and individually indicated to be incorporated by reference. Various changes and equivalent substitutions can be made to the various embodiments disclosed herein without departing from the true spirit and scope of the disclosure. Any feature, step or embodiment of an embodiment of the present disclosure can be used in combination with any other feature, step or embodiment, unless otherwise stated in the context.

SEQUENCE LISTING

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<120> Pharmaceutically acceptable salts of polypeptides and use thereof

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Ser Ile Glu Ser Asp Val
 20

Claims

1. A pharmaceutically acceptable salt of a polypeptide, wherein the polypeptide comprises the amino acid sequence YEKLLDTEI (SEQ ID NO: 1) or a functional variant thereof.
2. The pharmaceutically acceptable salt of a polypeptide according to claim 1, wherein the functional variant is a variant generated by one or more conservative substitutions in the LDTEI segment of SEQ ID NO: 1, and preferably, the conservative substitution is selected from the group consisting of a substitution between D and E, a substitution among L, V and I, and a substitution between T and S.
3. The pharmaceutically acceptable salt of a polypeptide according to claim 2, wherein the functional variant is a variant generated by replacing the LDTEI segment of SEQ ID NO: 1 with a sequence selected from the group consisting of LDTEL, LDTEV, LDTDI, LDTD L, LDTDV, LDSEI, LDSEL, LDSEV, LDS DI, LDS DL, LDS DV, LETEI, LETEL, LETEV, LETDI, LETDL, LETDV, VDTEI, VDTEL, VDTEV, VDTDI, VDTD L, VDTDV, IDTEI, IDTEL, IDTEV, IDTDI, IDTDL, IDTDV, IETEI, IETEL, IETEV, IETDI, IETDL, and IETDV.
4. The pharmaceutically acceptable salt of a polypeptide according to any one of the preceding claims, wherein the polypeptide is a chimeric peptide comprising an active peptide moiety and an internalization peptide moiety, and wherein the active peptide moiety is the amino acid sequence YEKLLDTEI (SEQ ID NO: 1) or a functional variant thereof, and the internalization peptide moiety is capable of facilitating uptake of the chimeric peptide by a cell.
5. The pharmaceutically acceptable salt of a polypeptide according to claim 4, wherein the internalization peptide moiety comprises the amino acid sequence YGRKKRRQRRR (SEQ ID NO: 2).
6. The pharmaceutically acceptable salt of a polypeptide according to claim 5, wherein the chimeric peptide comprises the amino acid sequence YGRKKRRQRRRYEKLLDTEI (SEQ ID NO: 3).
7. The pharmaceutically acceptable salt of a polypeptide according to any one of the preceding claims, wherein the salt is selected from the group consisting of a trifluoroacetate, an acetate, a hydrochloride and a phosphate.
8. A pharmaceutical composition comprising the pharmaceutically acceptable salt of a polypeptide according to any one of claims 1 to 7, and a pharmaceutically acceptable carrier, excipient and/or diluent.
9. The pharmaceutical composition according to claim 8, wherein the pharmaceutical composition is a pre-lyophilized formulation, preferably comprising histidine and trehalose.
10. The pharmaceutical composition according to claim 8, wherein the pharmaceutical composition is a lyophilized formulation, preferably prepared by lyophilizing the pre-lyophilized formulation of claim 9.
11. The pharmaceutical composition according to claim 8, wherein the pharmaceutical composition is a reconstituted formulation, preferably prepared by combining the lyophilized formulation of claim 10 with an aqueous solution.
12. The pharmaceutical composition according to any one of claims 8 to 11, for use in the treatment, amelioration or

prevention of a nervous system injury, a disease or pain associated with a nervous system injury, a neurodegenerative disease, anxiety or epilepsy in a subject, or for use as a neuroprotective agent.

- 5 13. A method for treating, ameliorating or preventing a nervous system injury, a disease or pain associated with a nervous system injury, a neurodegenerative disease, anxiety or epilepsy in a subject, comprising administering to the subject the pharmaceutically acceptable salt of a polypeptide according to any one of claims 1-7, or the pharmaceutical composition according to any one of claims 8 to 12.
- 10 14. Use of the pharmaceutically acceptable salt of a polypeptide according to any one of claims 1-7, or the pharmaceutical composition according to any one of claims 8 to 12 in the preparation of a medicament for the treatment, amelioration or prevention of a nervous system injury, a disease or pain associated with a nervous system injury, a neurodegenerative disease, anxiety or epilepsy in a subject, or in the preparation of a neuroprotective agent.
- 15 15. The pharmaceutical composition of claim 12, the method of claim 13, or the use of claim 14, wherein the nervous system injury is a nervous system injury caused by excitatory neurotoxicity.
- 20 16. The pharmaceutical composition, the method or the use of 15, wherein the nervous system injury caused by excitatory neurotoxicity comprises an injury selected from the group consisting of a stroke, a spinal cord injury, an ischemic or traumatic injury to a brain or spinal cord, an injury to a neuron in central nervous system (CNS) including an acute CNS injury, an ischemic stroke or a spinal cord injury, a hypoxia, ischemia, or mechanical injury and an injury caused by a neurodegenerative disease, anxiety, epilepsy or stroke.
- 25 17. The pharmaceutical composition of claim 12, the method of claim 13, or the use of claim 14, wherein the neurodegenerative disease is selected from the group consisting of Alzheimer's disease, amyotrophic lateral sclerosis (ALS), Parkinson's disease and Huntington's disease.
- 30 18. The pharmaceutical composition of claim 12, the method of claim 13, or the use of claim 14, wherein the nervous system injury or pain is located in the peripheral nervous system or the central nervous system.
- 35 19. The pharmaceutical composition of claim 12, the method of claim 13, or the use of claim 14, wherein the disease associated with a nervous system injury is a stroke.
- 40 20. The pharmaceutical composition, method or use of claim 19, wherein the stroke is selected from the group consisting of an ischemic stroke, a hemorrhagic stroke, and a hemorrhagic stroke converted from an ischemic stroke.
- 45 21. The pharmaceutical composition, method or use of claim 20, wherein the stroke is an ischemic stroke.
- 50
- 55

Figure1

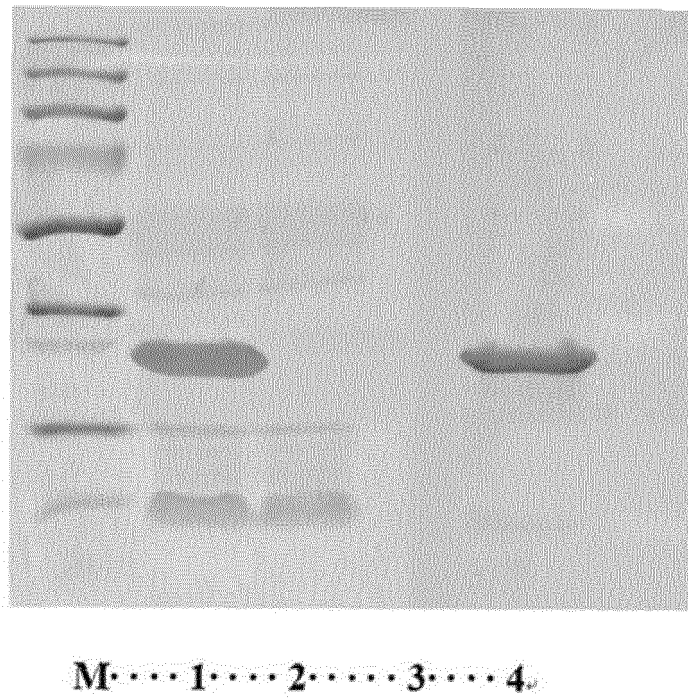


Figure 2

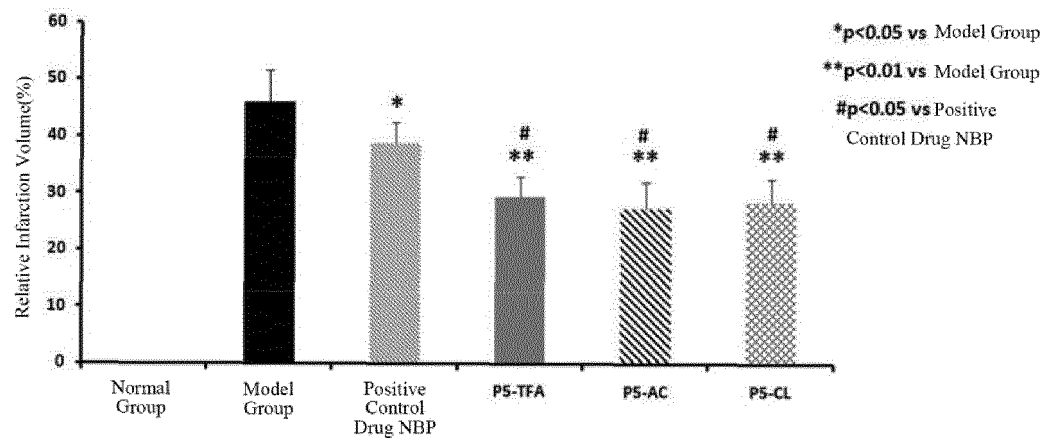


Figure 3

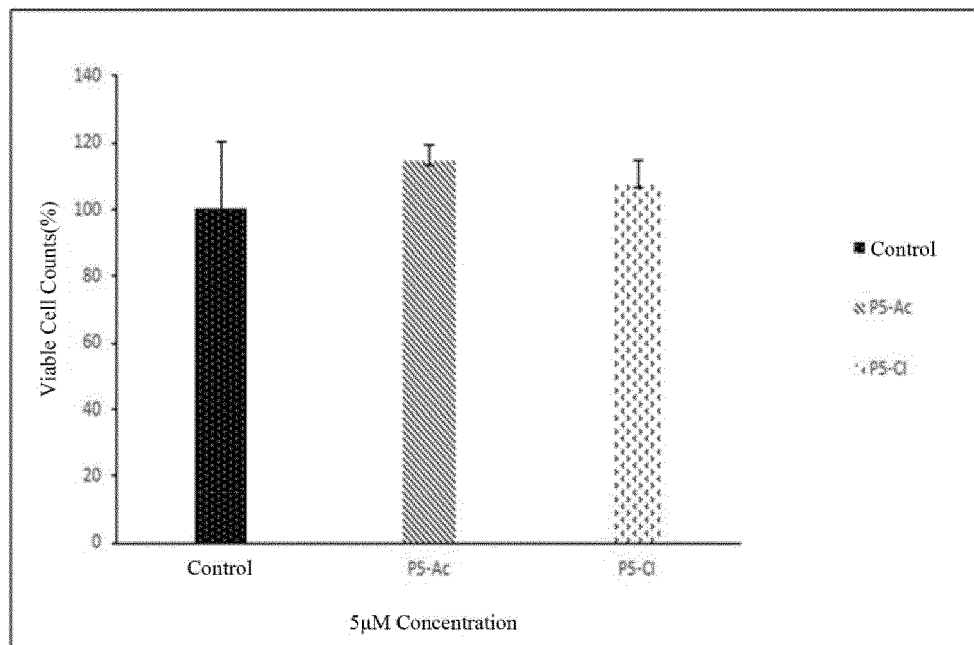
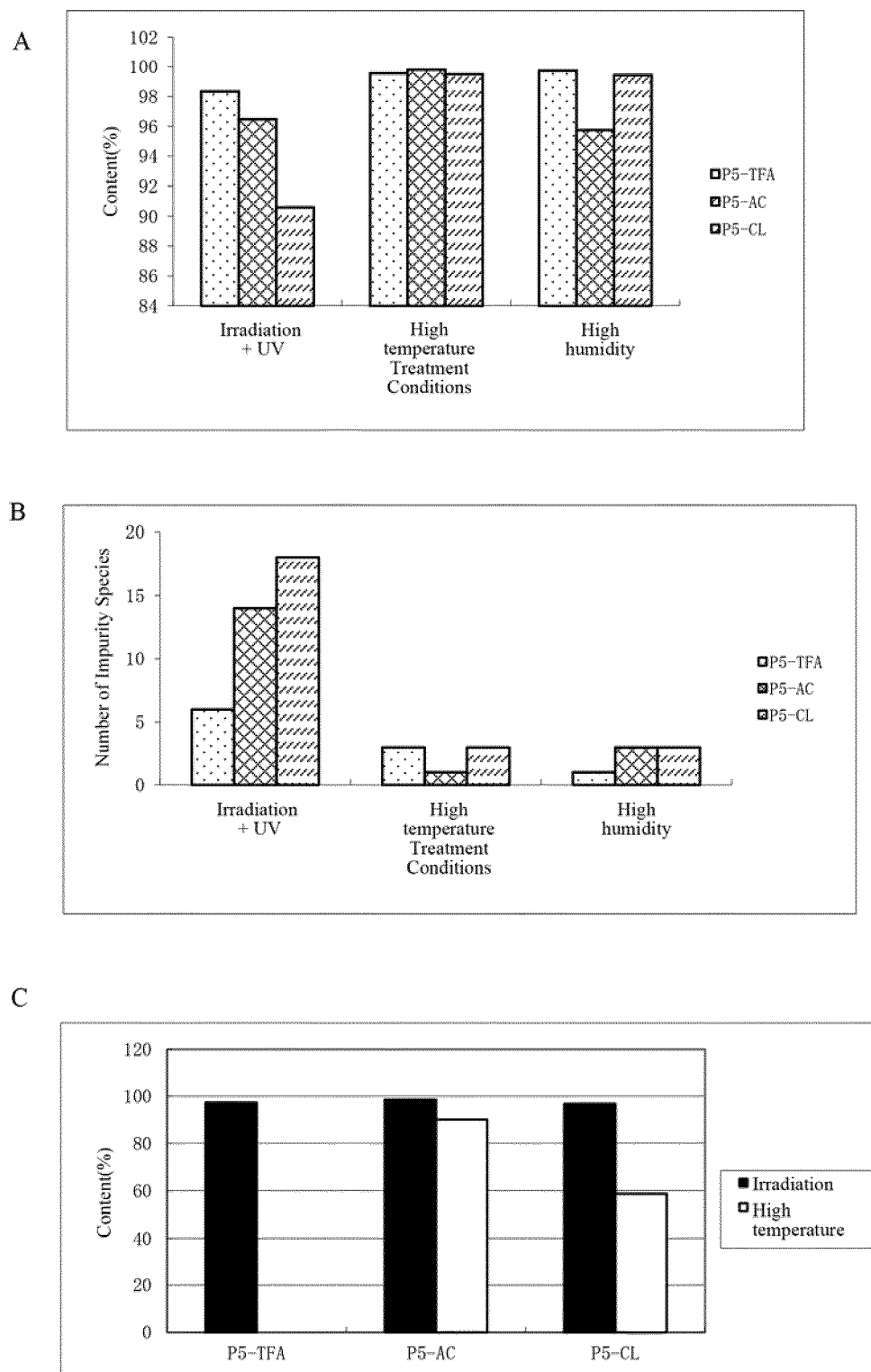
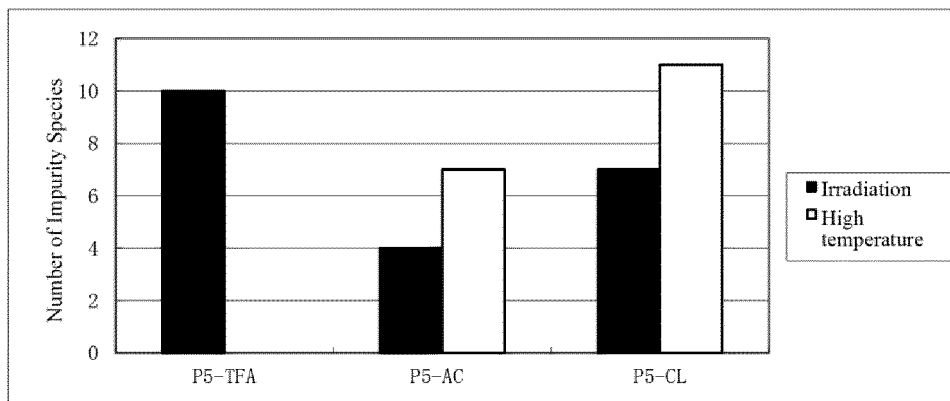


Figure 4



D



INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2017/091792

A. CLASSIFICATION OF SUBJECT MATTER

C07K 7/06 (2006.01) i; C07K 19/00 (2006.01) i; A61K 38/08 (2006.01) i; A61K 47/42 (2017.01) i; A61P 25/00 (2006.01) i; A61P 29/00 (2006.01) i; A61P 25/28 (2006.01) i; A61P 25/22 (2006.01) i; A61P 25/08 (2006.01) i; A61P 9/10 (2006.01) i; A61P 21/00 (2006.01) i; A61P 25/02 (2006.01) i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K; A61K; A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS, CNKI, CNTXT, DWPI, SIPOABS, EPTXT, USTXT, WOTXT, JPTXT, NCBI, Chinese Patent Biological Sequence Retrieval System, Genbank, EMBL, STN and Searching Items: 突触后致密物-95 蛋白质, postsynaptic density-95, density protein receptor, PSD-95, 抑制剂, inhibitor, 活性肽, active peptide, 嵌合肽, chimeric peptide, Tyr Glu Lys Leu Leu Asp Thr Glu Ile, YEKLDDTEI, SEQID NOs: 2, 3, and the sequence of claim 3

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
E	WO 2017185249 A1 (BIOCELLS BEIJING BIOTECH CO., LTD.) 02 November 2017 (02.11.2017), claims 1-17	1-6, 8, 10, 12, 14, 15-21 (partly)
E	CN 107312069 A (BIOCELLS (BEIJING) BIOTECHNOLOGY CO., LTD.) 03 November 2017 (03.11.2017), claims 1-17	1-6, 8, 10, 12, 14, 15-21 (partly)
X	CN 101827604 A (NONO, INC.; ARBOR VITA CORPORATION) 08 September 2010 (08.09.2010), claims 1-39, and description, paragraphs [0048], [0146], [0147] and [0156]	1, 7-12, 14, 15-21 (partly)

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 20 March 2018	Date of mailing of the international search report 12 April 2018
Name and mailing address of the ISA State Intellectual Property Office of the P. R. China No. 6, Xitucheng Road, Jimenqiao Haidian District, Beijing 100088, China Facsimile No. (86-10) 62019451	Authorized officer WANG, Xiangyu Telephone No. (86-10) 62411992

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2017/091792

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 101827604 A (NONO, INC.; ARBOR VITA CORPORATION) 08 September 2010 (08.09.2010), entire document	2-6
X	GenBank Accession number XP_014940570, Version XP_014940570.1. GenBank. 15 December 2015 (15.12.2015), page 1	1-3, 7-11
A	GenBank Accession number XP_014940570, Version XP_014940570.1. GenBank. 15 December 2015 (15.12.2015), page 1	4-6, 12, 14, 15-21 (partly)
X	NIELSEN, H. B. et al. GenBank Accession number CDC10956, Version CDC10956.1. GenBank. 31 May 2013 (31.05.2013), page 1	1-3, 7-11
A	NIELSEN, H. B. et al. GenBank Accession number CDC10956, Version CDC10956.1. GenBank. 31 May 2013 (31.05.2013), page 1	4-6, 12, 14, 15-21 (partly)
X	LIN, K. et al. GenBank Accession number EXX70775, Version EXX70775.1. GenBank. 18 March 2014 (18.03.2014), page 1	1-3, 7-11
A	LIN, K. et al. GenBank Accession number EXX70775, Version EXX70775.1. GenBank. 18 March 2014 (18.03.2014), page 1	4-6, 12, 14, 15-21 (partly)
X	GenBank Accession number WP_062612638, Version WP_062612638.1. GenBank. 15 May 2016 (15.05.2016), page 1	1-3, 7-11
A	GenBank Accession number WP_062612638, Version WP_062612638.1. GenBank. 15 May 2016 (15.05.2016), page 1	4-6, 12, 14, 15-21 (partly)
X	GenBank Accession number WP_065739501, Version WP_065739501.1. GenBank. 27 July 2016 (27.07.2016), page 1	1-3, 7-11
A	GenBank Accession number WP_065739501, Version WP_065739501.1. GenBank. 27 July 2016 (27.07.2016), page 1	4-6, 12, 14, 15-21 (partly)
X	CN 103648518 A (ARBOR VITA CORP.) 19 March 2014 (19.03.2014), claims 12, 27 and 32, and description, paragraphs [0045], [0050], [0072] and [0119]	1, 7-12, 14, 15-21 (partly)
A	CN 103648518 A (ARBOR VITA CORP.) 19 March 2014 (19.03.2014), entire document	2-6

Form PCT/ISA /210 (continuation of second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CN2017/091792

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:13 and 15-21 (partly)
because they relate to subject matter not required to be searched by this Authority, namely:
[1] PCT Rule 39.1 (iv)-methods for treatment of the human or animal body by surgery or therapy.
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
 Information on patent family members

 International application No.
 PCT/CN2017/091792

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
WO 2017185249 A1	02 November 2017	None	
CN 107312069 A	03 November 2017	None	
CN 101827604 A	08 September 2010	US 2009036376 A1	05 February 2009
		AU 2008272903 A8	06 May 2010
		DK 2175873 T3	14 December 2015
		JP 2014129353 A	10 July 2014
		AU 2008272903 B2	29 August 2013
		KR 101604428 B1	17 March 2016
		CA 2692412 A1	08 January 2009
		KR 20100038366 A	14 April 2010
		WO 2009006548 A2	08 January 2009
		WO 2009006548 A3	19 March 2009
		EP 2175873 A2	21 April 2010
		AU 2008272903 A1	08 January 2009
		ES 2555172 T3	29 December 2015
		US 8536129 B2	17 September 2013
		US 8008253 B2	30 August 2011
		EP 2175873 A4	05 October 2011
		JP 5775303 B2	09 September 2015
		JP 2010532761 A	14 October 2010
		CA 2692412 C	07 February 2017
		US 2012083449 A1	05 April 2012
		CN 101827604 B	07 May 2014
		EP 2175873 B1	11 November 2015
CN 103648518 A	19 March 2014	WO 2012176172 A2	27 December 2012
		KR 20140053971 A	08 May 2014
		EP 2723363 A2	30 April 2014
		AU 2012274910 A1	16 January 2014

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2017/091792

Patent Documents referred in the Report	Publication Date	Patent Family	Publication Date
		WO 2012176172 A3	28 March 2013
		US 2014248257 A1	04 September 2014
		EP 2723363 A4	10 December 2014
		JP 2017193535 A	26 October 2017
		CN 103648518 B	16 November 2016
		CA 2839630 A1	27 December 2012
		AU 2012274910 B2	11 May 2017
		JP 2014520142 A	21 August 2014

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Non-patent literature cited in the description

- **S. S. WONG.** Chemistry of Protein Conjugation and Cross-Linking. CRC Press, 1991 **[0044]**
- Organic Syntheses Collective Volumes. Mol. Biotechnol. John Wiley & Sons, Inc, 1998, vol. 9, 205-223 **[0045]**
- **HRUBY.** *Curr. Opin. Chem. Biol.*, 1997, vol. 1, 114-119 **[0045]**
- **OSTERGAARD.** *Mol. Divers.*, 1997, vol. 3, 17-27 **[0045]**
- **OSTRESH.** *Methods Enzymol.*, 1996, vol. 267, 220-234 **[0045]**
- **LEES et al.** *N Engl J Med*, 2006, vol. 354, 588-600 **[0061]**
- **FINGL et al.** The Pharmacological Basis of Therapeutics. 1975, 1 **[0064]**
- Rankin Stroke Outcome Scale. Rankin, Scott MedJ. 1957, vol. 2, 200-15 **[0071]**
- **MAHONEY et al.** *Maryland State Medical Journal*, 1965, vol. 14, 56-61 **[0072]**